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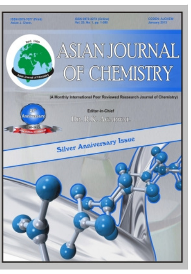

HPLC Determination of Chlorophenols in Water with Separation by Ionic Liquids [Bmim]BF_4 Aqueous Two-phase Solvent Sublation

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2,4-Dichlorophenol, 2,6-dichlorophenol and 4-chlorophenol were separated from water sample and enriched by ionic liquids [Bmim]BF_4 aqueous two-phase solvent sublation with [Bmim]BF_4 as solvent from the water sample at pH 10.70 and flotation with nitrogen at a flow rate of 35 mL min^{-1}, for 40 min. At the end of sublation, 10 μL of the supernatant ionic liquids phase were taken for HPLC analysis. Detection limits (3s/b) of three chlorophenols were 15, 22 and 10 μg L^{-1}, respectively. Tests for recovery were made with a blank water sample as matrix by standard addition method, values of recovery were found in the range of 90-102 %, with values of RSD's (n = 11) ranged from 1.5 % to 4.3 %.

Key Words: Ionic liquids aqueous two-phase, Solvent sublation, HPLC, Chlorophenols.

INTRODUCTION

Chlorophenols (CPs) are a class of compounds with benzene ring of phenol connected with chlorine atoms. Chlorophenols as commonly used chemical raw materials, are often used as pesticides, preservatives, herbicides and they are a category of widespread organic contaminants. Because chlorophenols are high toxicity, difficult biodegradation, the carcinogenicity-mutagenicity-teratogenicity and may concentrate through the food chain in organism, have been listed the priority pollutant ranks by environmental protection agency of various countries[1,2].

At present, the detection methods of chlorophenols are mainly high performance liquid chromatography (HPLC)[3], ultraviolet spectrophotometry (UV)[4], gas chromatography (GC)[5,6] and gas chromatography-mass spectrometry (GC-MS)[7,8]. The methods of sample preparation are mainly solvent extraction[9], solid phase extraction[10,11], solid phase microextraction[12,13], headspace solid phase extraction[14], liquid phase microextraction[15] and liquid-liquid extraction[16].

Ionic liquids aqueous two-phase system (ILATPS) is a new system which can be used for extraction of substances in recent years[17]. Li *et al.*[18] extracted trace roxithromycin in real water samples by use of ionic liquid([Bmim]BF_4)-salt(Na_2CO_3) aqueous two-phase system, the average extraction efficiency is up to 90.7 %. He *et al.*[19] extracted testosterone and epitestosterone in human urine using aqueous two-phase systems of ionic liquid([Bmim]Cl) and salt(K_2HPO_4), the extraction efficiencies for both analytes were 80-90 % in a one-step extraction. Li *et al.*[20] extracted opium alkaloids in pericarpium papaveris by ionic liquid([Bmim]Cl)-salt(K_2HPO_4) aqueous two-phase system, the recoveries were 99.3-102.0 %. Du *et al.*[21] extracted directly proteins from human body fluids by ionic liquid aqueous two-phase system([Bmim]Cl/K_2HPO_4) for the first time, enrichment factor is 20.

Solvent sublation[22] is an adsorptive bubble separation process that organic solvent is on the top of the aqueous phase. When some kind of inert gas (nitrogen, air and carbon dioxide are generally) move through the column, the innumerable small gas bubbles are formed. The components, which to be separated are adsorbed by the gas bubble. The polar end (S-) is orientation arrange in aqueous solution and the non-polar end(R-) is orientation arrange in gas bubbles. The target molecules are carried to the top phase by the rising bubbles, dissolved in organic solvent, which is insoluble in water, or suspended in two-phase interface (Fig. 1). The process takes advantage of good selectivity, high sensitivity, low detection limit, great enrichment factors[23], big amount of treating samples, no emulsification[24] and less organic solvent consumption. It has been widely used in tetracycline antibiotics[25], butyl acetate[26] and penicillin G[27]. But the chlorophenols of environmental hormone have not be applied by solvent sublation, the mechanism should also be researched.

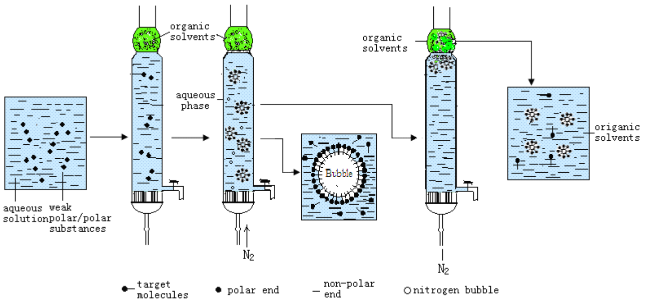

Fig. 1. Basic principle of gas solvent sublation

In this paper, 2,4-dichlorophenol, 2,6-dichlorophenol and 4-chlorophenol in water were simultaneously determined by solvent sublation coupled to ionic liquids aqueous two-phase extraction with [Bmim]BF_4 as solvent, which could not cause to secondary pollution of environment because benzene, toluene, xylene, isoamyl alcohol and so on were not used as sublation reagent in the solvent sublation process. A new system of ionic liquids aqueous two-phase solvent sublation for separation/enrichment was established which followed by high performance liquid chromatography.

EXPERIMENTAL

Agilent 1100 Series high performance liquid chromatography was used for separation and determination of analytes. A UV-2550 ultraviolet obvious spectrophotometer (Shimadzu Instruments Co. Ltd.) was used for optimizing the parameters of the solvent sublation. A pHS-4 Intelligent pH meter (Jiangsu Jiangfen Electro Analytical Instrument Co. Ltd.) was used for pH measurements. BN0828 electronic analytical balance (Shanghai Precision Scientific Instrument Co. Ltd. China Bridge) was used for measuring reagents.

Ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim]BF_4) was purchased from Shanghai Cheng Jie Chemical Company. Acetonitrile (ACN), Na_2CO_3, $NaHCO_3$, HCl and NaCl were purchased from Shenyang Sinopharm. 2,4-dichlorophenol (2,4-DCP), 2,6-dichlorophenol(2,6-DCP) and 4-chlorophenol (4-CP) were obtained from Aladdin.

Standard solutions (1.0×10^{-4} mol L^{-1}) of each chlorophenols were prepared in a 250 mL measuring flask with proper amount of deionized water. 1 mL of each standard solutions was put in 100 mL measuring flask by adding Na_2CO_3 solution to make 1.0×10^{-6} mol L^{-1} of mixed standard solution. All solutions were stored at 4 ºC in the refrigerator. Acetonitrile was chromatographic pure reagent and the others were analytical reagents. The deionized water was used in the experiment.

1 mL Chlorophenols were accurately shift from each standard solutions to 100 mL beaker and 0.25 g mL^{-1} Na_2CO_3 solution was added. Then the pH of the solution was adjusted to 10.70 with $NaHCO_3$ solution. The solution was stirred uniformly and then transferred to a solvent sublation column. The chlorophenols were floated by N_2 bubble at a flow rate of 35 mL min^{-1} from the bottom of the column for 40 min and extracted into 5 mL organic phase of [Bmim]BF_4: acetonitrile (v:v = 3:2) on the surface of the aqueous solution. After stopping and stable, the top phase of mixture was sucked into 5 mL measuring flask by adding water to the scale. UV-VIS spectrometry was more economical, rapid, simple and convenient than HPLC. So, the conditions of solvent sublation were optimized by UV-VIS spectrometry. Then, 10 µL solution containing analytes was determined by HPLC under chromatographic conditions.

Detection method: Chromatographic separations were carried out using a Kromasil 100 C_{18} chromatographic column (150 mm × 4.6 mm × 5 µm) at a flow rate of 1.0 mL min^{-1} at 30 ºC of column temperature. The mobile phase was the mixed solution of acetonitrile and water ($V_{ACN} : V_{water} = 60:40$). The detection wavelength were 292 nm, 300 nm and 280 nm for 2,4-dichlorophenol, 2,6-dichlorophenol and 4-chlorophenol, respectively. The column pressure was 43 bar and the injection volume was 10 µL.

RESULTS AND DISCUSSION

Effect of the amount of Na_2CO_3: Na_2CO_3 was chose as salting-out agent of [Bmim]BF_4 aqueous two-phase. Simultaneously, effect of amount of Na_2CO_3 was studied. 1 mL chlorophenols were respectively sucked for solvent sublation by the experimental method. The top phase after solvent sublation was taken for determination by UV-VIS. It was clear that the absorbance of [Bmim]BF_4-rich top phase after solvent sublation increased gradually and tended to be stable finally with adding salting-out agent Na_2CO_3 from Fig. 2. It also indicated that separation of two phases was more and more obvious with the increased amount of Na_2CO_3. The absorbance reached the maximum value when the amount of Na_2CO_3 was 0.25 g mL^{-1}. It illuminated that the amount of salting-out not only made for the complete separation of two phases, but also made system solution achieve the optimum ionic strength and be propitious to solvent sublation. So the amount of Na_2CO_3 used for forming two phases was 0.25 g mL^{-1}.

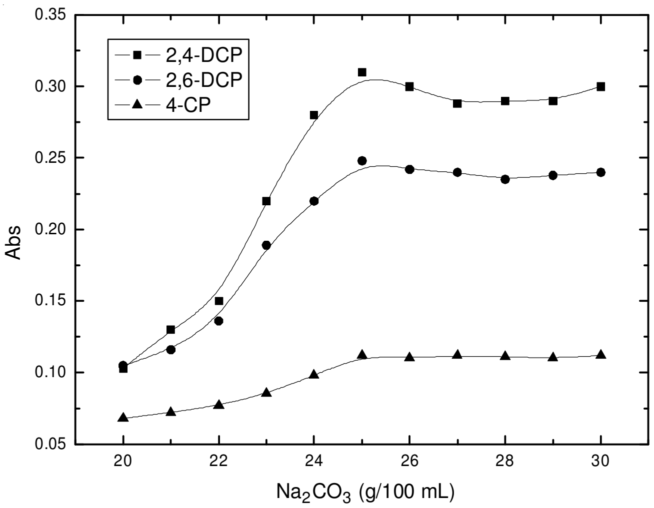

Fig. 2. Effects of the amount of Na_2CO_3 on the sublation yield of three chlorophenols (■ 2,4-DCP; ● 2,6-DCP; ▲ 4-CP)

Solvent of decreasing the viscosity of ionic liquid: Because the viscosity and surface tension of ionic liquid are great, the floating bubble was unable to penetrate through the surface of two phases and dissolve into ionic liquid phase. The bubbles directly overflow from inside surface of container between ionic liquid and inwall of column. Three chlorophenols, which were absorbed by the bubble could not dissolve in ionic liquid phase and then returned to water phase with the broken bubble. So ionic liquid can not be directly used for solvent sublation. In this experiment, the solvent which the density is lower than water and it can be dissolved with ionic liquid to form aqueous two-phase was added in ionic liquid. But the solvent to be chosen are fewer, acetonitrile was found to be fit for this experiment. Therefore, acetonitrile was selected as diluting agent to decrease viscosity of ionic liquid. The results show that acetonitrile was added in ionic liquid could increase the absorbance of [Bmim]BF_4-rich top phase after solvent sublation. This indicated that viscosity and density were reduced after adding acetonitrile in ionic liquid and it was propitious to solvent sublation. The absorbance began to decrease when the ratio of ionic liquid to ACN is more than 1.6 (Fig. 3). It was clear that V_{RTIL}:V_{ACN}= 3:2 was the best.

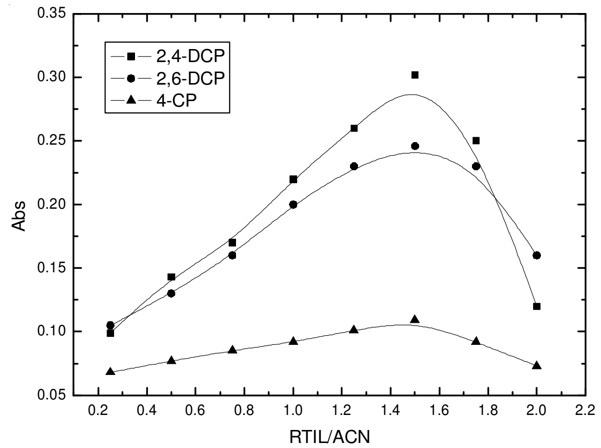

Fig. 3. Effects of RTIL/acetonitrile; (■ 2,4-DCP; ● 2,6-DCP; ▲ 4-CP)

Effect of pH: Various pH have effect on solvent sublation. Effects of pH from 10.4 to 11.4 on sublation efficiencies of three chlorophenols were investigated. It can be seen that the absorbance of [Bmim]BF_4-rich top phase after solvent sublation increased gradually and decreased gradually after achieve the maximum from Fig. 4. Absorbance of 2,4-dichlorophenol and 2,6-dichlorophenol reached the peak when pH was 10.70. Absorbance of 4-chlorophenol reached the peak when pH was 10.80. So pH 10.70 of the solution was chosen in this experiment.

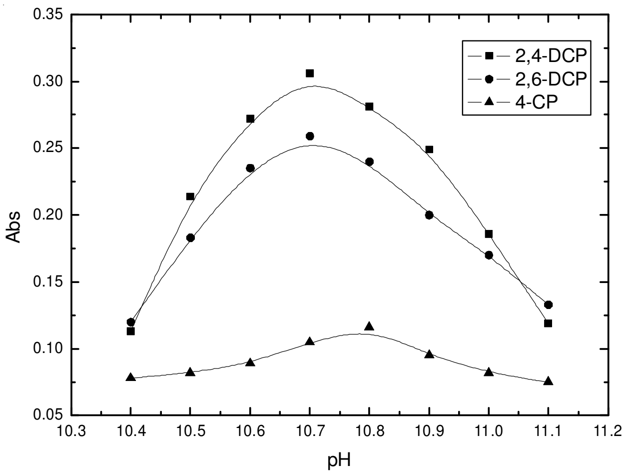

Fig. 4. Effects of pH; (■ 2,4-DCP;   2,6-DCP; ▲ 4-CP)

Effect of gas flow rate: Gas flow rate was changed and the other conditions were performed according to experimental method. As is shown in Fig. 5, rising the gas flow rate from 10 mL min^{-1} to 50 mL min^{-1} lead to the improvement of the sublation efficiencies for three chlorophenols. However, when the gas flow was over 35 mL min^{-1}, the reduction of sublation efficiency was observed. Because gas flow rate was too quick, the analytes on the bubble were easily broken off. From various experimental results, the gas flow rate was fixed at 35 mL min^{-1} for this experiment.

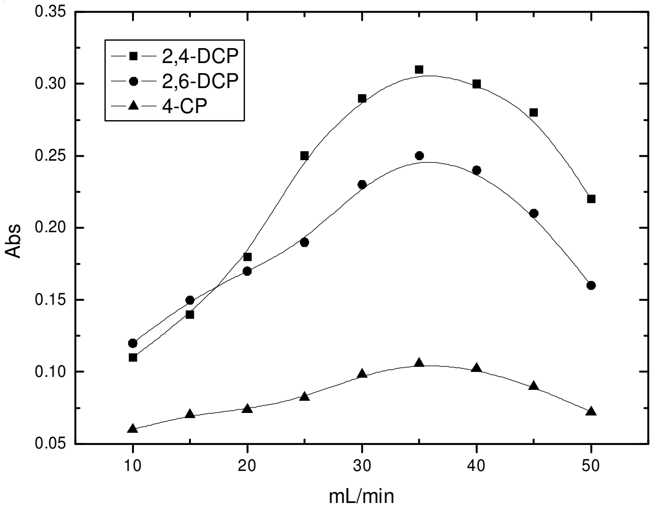

Fig. 5. Effects of sublation gas flow rate; (■ 2,4-DCP; ● 2,6-DCP; ▲ 4-CP)

Effect of sublation time: Sublation time was changed and the other conditions were executed according to experimental method. Fig. 6 was shown that the absorbance of the top phase after solvent sublation was the highest when sublation time was 40 min. If sublation time was continually increased, the absorbance changed rarely because the solvent sublation basically achieved a balance. The sublation time was fixed at 40 min in this experiment.

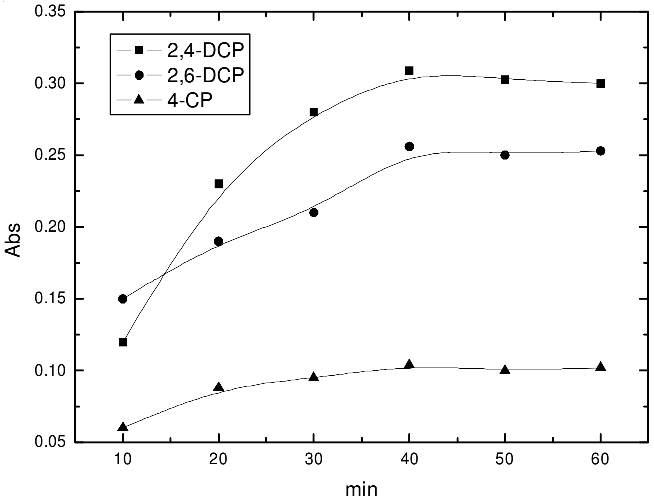

Fig. 6. Effects of sublation time; (■ 2,4-DCP; ● 2,6-DCP; ▲ 4-CP)

Effect of interfering substances: Under the optimal experimental conditions, the influences of many coexisting substances were determined which the relative error was less than ± 5 %. The experimental results (μg mL^{-1}) indicated that Na$^+$, NO$_3^-$, CO$_3^{2-}$, HCO$_3^-$, SO$_4^{2-}$, Cl$^-$, Br$^-$, K$^+$, Zn^{2+}, Fe^{3+} and Al^{3+} were no interference; Mg^{2+} and Ca^{2+} had interference on the test; Cu^{2+}, Cd^{2+}, Pb^{2+}, F$^-$ had serious interference; 2-CP, 2,4,6-TCP, phenol and so on were not big influence to the experimental result (Table-1).

TABLE-1
EFFECT OF COEXISTENCE SUBSTANCES

Coexistence substances	Addition	Coexistence substances	Addition
Na$^+$	500	Cu^{2+}	1.0
K$^+$	700	Cd^{2+}	4.0
Zn^{2+}	250	Pb^{2+}	2.5
Fe^{3+}	500	F$^-$	1.0
Al^{3+}	700	2-CP	50
NO$_3^-$	500	2,3-DCP	68
CO$_3^{2-}$	500	3,5-DCP	72
SO$_4^{2-}$	500	2,4,6-TCP	70
HCO$_3^-$	150	2,3,4-TCP	58
Cl$^-$	500	2,3,5,6-TeCP	66
Br$^-$	500	PCP	60
Mg^{2+}	100	Phenol	55
Ca^{2+}	100	o-Dihydroxybenzene	65

Sublation effect: Sublation efficiency, partition coefficient and enrichment factor were calculated by the following formula. Sublation efficiency were 80 to 90 %, partition coefficient were 400 and enrichment factor were 80.

$$\text{Sublation efficiency: } E = C_tV_t/C_0V_b$$

$$\text{Partition coefficient: } K = C_t/C_b$$

$$\text{Enrichment factor: } \alpha = C_t/C_0.$$

where C_0 is the total concentration of chlorophenols; C_t is the concentration of chlorophenols in [Bmim]BF$_4$-acetonitrile phase after sublation; C_b is the concentration of chlorophenols in aqueous phase after sublation; V_t is volume of top phase; V_b is volume of bottom phase.

Detection limits and correlation coefficients: A series of mixed standard solution containing three chlorophenols were made up. Solvent sublation was carried out under the optimal experimental conditions. The top phase after solvent sublation was determined under the selected chromatographic conditions. Calibration curves were drafted by the measured peak area and the concentration of each component in the mixed standard solution. Correlation coefficients of 2,4-DCP, 2,6-DCP, 4-CP were 0.9997, 0.9991 and 0.9989, respectively. Detection limits of 2,4-DCP, 2,6-DCP, 4-CP at a signal to noise ratio of 3 were 15, 22 and 10 μg L^{-1}, respectively.

Addition standard recovery and precision: Experiment of standard additions was performed by water samples, which did not determined 2,4-DCP, 2,6-DCP and 4-CP. 0.1, 0.2 and 0.5 μg L^{-1} of mixed standard solution containing three chlorophenols were added in the system. Each concentration was parallel determined 5 times. The samples were collected by experimental method and analyzed under the selected chromatographic conditions after solvent sublation which was performed under the optimal experimental conditions. The chromatograms for standard solution, a blank sample and the spike sample were shown in Figs. 7-9. Retention times of 2,4-DCP, 2,6-DCP, 4-CP were 3.091, 2.864 and 2.394 min, respectively. Average recoveries (n = 5) were 90-102 %, relative standard deviation (RSD) were 1.5-4.3 %. It can be seen that the proposed method is good precision and high recovery. The results were shown in Table-2.

TABLE-2
AVERAGE RECOVERY OF STANDARD ADDITIONS (n = 5)

Chlorophenols	Added (μg L^{-1})	Measured (μg L^{-1})	Average recovery (%)	RSD (%)
2,4-Dichlorophenol	0.100	0.090	90	2.1
	0.200	0.190		1.5
	0.500	0.425		3.6
2,6-Dichlorophenol	0.100	0.103	102	1.2
	0.200	0.204		4.1
	0.500	0.505		3.4
4-Chlorophenol	0.100	0.098	97	4.3
	0.200	0.198		3.7
	0.500	0.047		2.9

Sample determination: Eight samples were taken from waste water of paper mill, waste water of chemical industry, river water, lake water, rain water, tap water, water of deep well and mineral water were determined by experimental method. 2,4-Dichlorophenol was determined in waste water of paper mill and chemical industry. The concentration were 450 μg L^{-1} and 700 μg L^{-1}, respectively. Three chlorophenols were not determined in the other samples.

Extraction mechanism and IR spectrum analyses: For discussing the interreaction three chlorophenols with

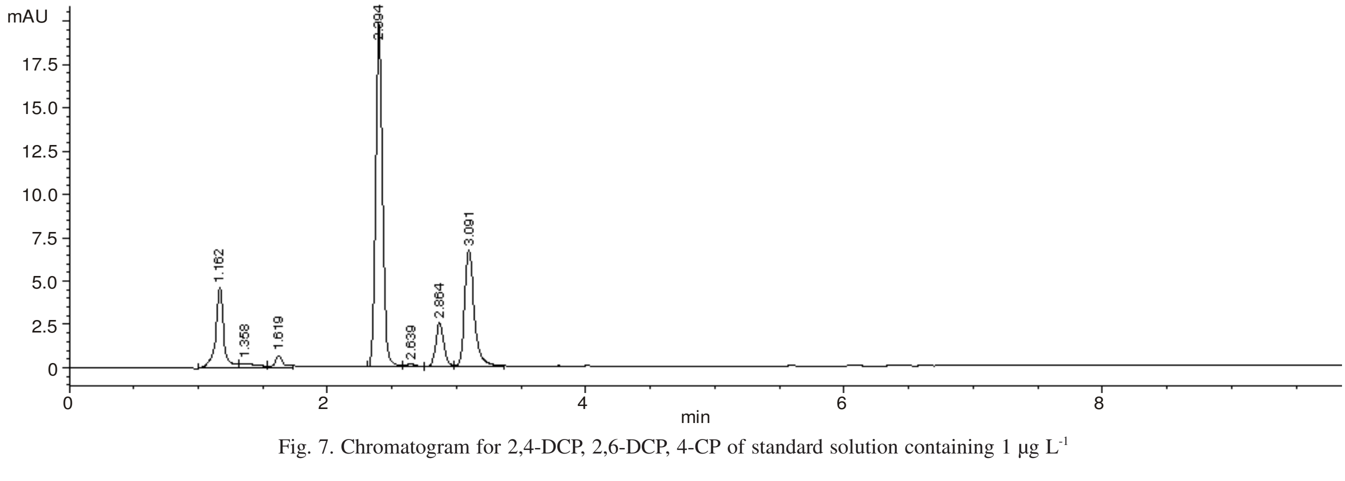

Fig. 7. Chromatogram for 2,4-DCP, 2,6-DCP, 4-CP of standard solution containing 1 μg L^{-1}

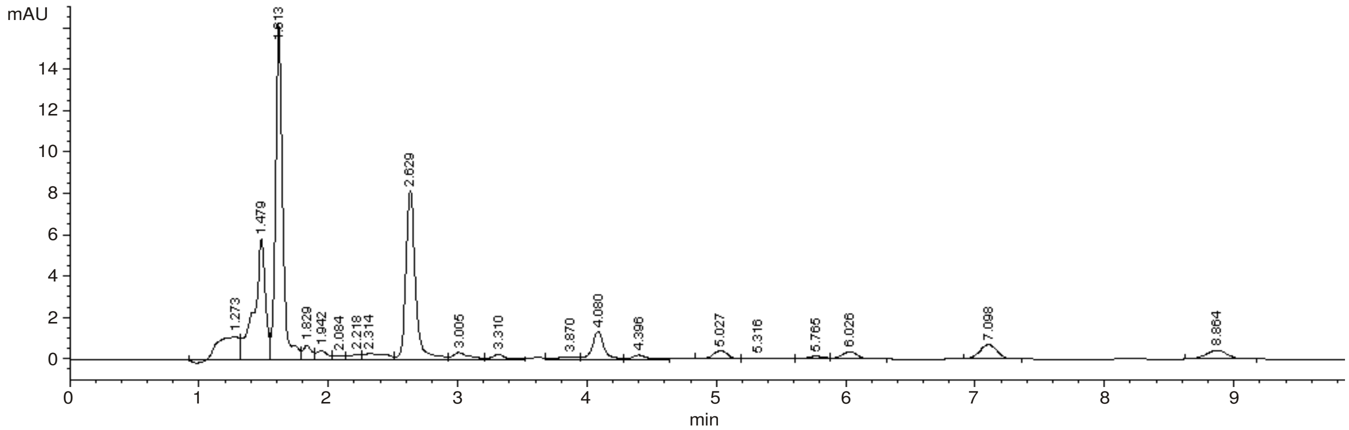

Fig. 8. Chromatogram of a blank sample

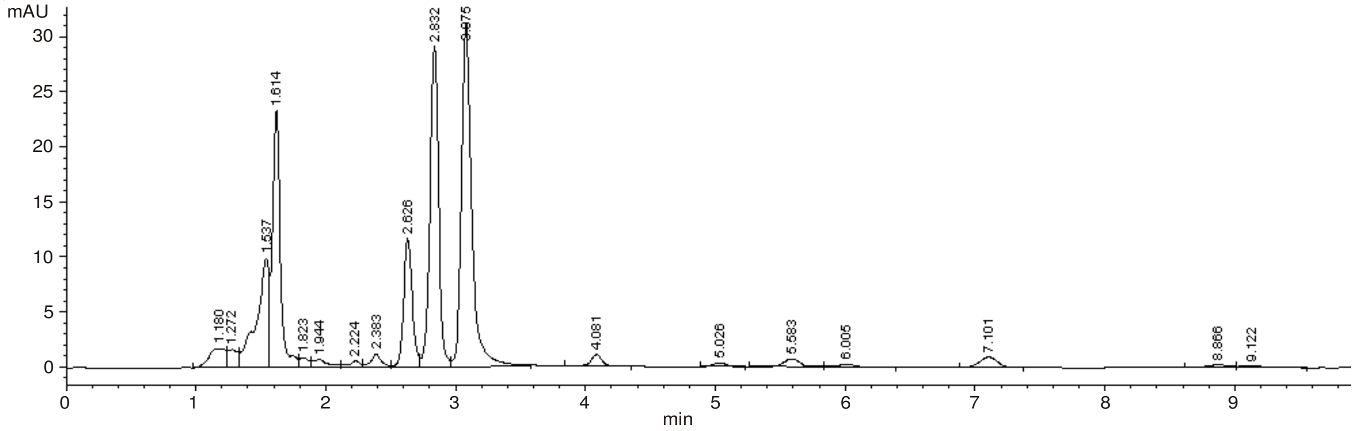

Fig. 9. Chromatogram for 2,4-DCP, 2,6-DCP, 4-CP of spike sample

[Bmim]BF$_4$-acetonitrile, the IR spectrums for various groups of three chlorophenols and [Bmim]BF$_4$-acetonitrile were illustrated in Figs. 10-12. Contrasting with three IR spectrums, it was obvious that the B-F of ionic liquid [Bmim]BF$_4$-acetonitrile existed stretching vibrations, which moved from 1032 cm^{-1} to 1083 cm^{-1} and absorption peak became wide. From this, it can be decided that O-H of three chlorophenols and B-F of [Bmim]BF$_4$ had formed hydrogen bond in the sublation. So three chlorophenols could be sublated into [Bmim]BF$_4$ phase. Ionic liquid was only solvent and didn't react with three chlorophenols in the sublation.

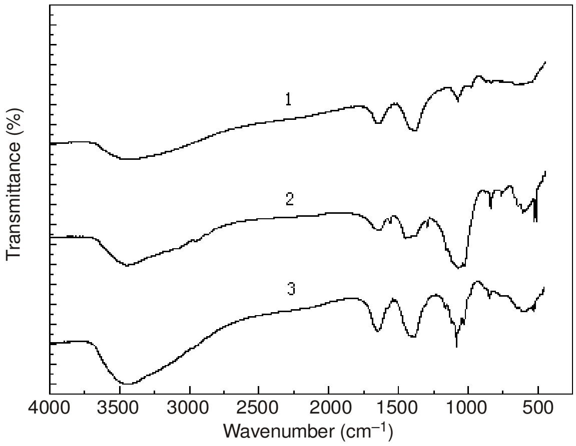

Fig. 10. IR spectrum of 2,4-DCP, RTIL and [Bmim]BF$_4$-rich phase; 1) 2,4-DCP, 2) 2,4-DCP + [Bmim]BF$_4$-ACN, 3) [Bmim]BF$_4$-ACN

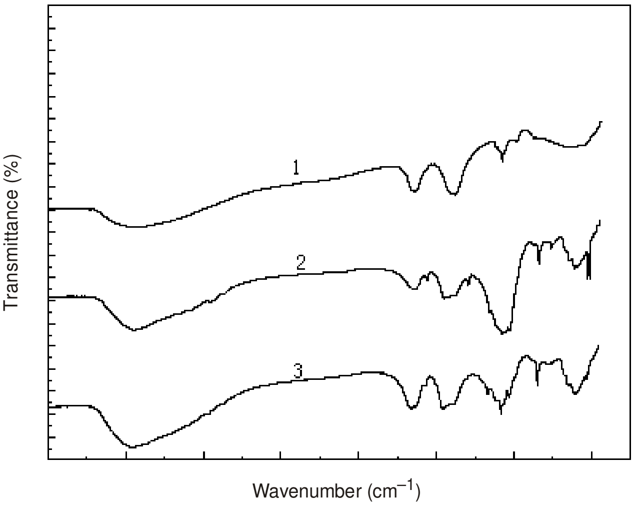

Fig. 11. IR spectrum of 2,6-DCP, RTIL and [Bmim]BF\-rich phase; 1) 2,6-DCP, 2) 2,6-DCP+[Bmim]BF$_4$-ACN, 3) [Bmim]BF$_4$-ACN

Conclusion

2,4-Dichlorophenol, 2,6-dichlorophenol and 4-chlorophenol were simultaneously determined by ionic liquids aqueous two-phase solvent sublation coupled with HPLC in this study. The proposed method is large volume of treating samples, no emulsification, good precision, great enrichment factors and high recovery.

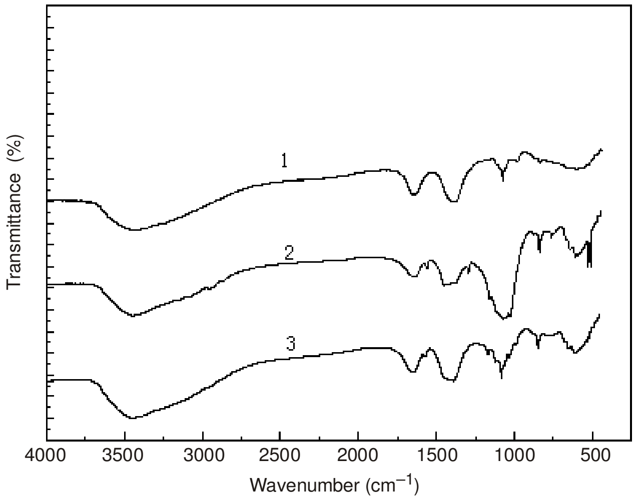

Fig. 12. IR spectrum of 4-CP, RTIL and [Bmim]BF$_4$-rich phase; 1) 4-CP, 2) 4-CP+[Bmim]BF$_4$-ACN, 3) [Bmim]BF$_4$-ACN

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